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"Research in Photosynthesis"

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RESEARCH IN PHOTOSYNTHESIS

Introduction

Contract No. NASw-747 has supported in whole or in part, a number of investigations concerning photosynthesis or photosynthetic material. This quarterly progress report presents a brief survey of the progress of each of these investigations over the past two years of effort, and notes references to publications which have resulted from these studies. Included in these surveys are summaries of work performed during the current quarter.

Chloroplast Lipids^(1,2)

Work in the last year in the area of plant lipids is summarized in the above publications. Although this work does not directly contribute to an understanding of the manner in which the amphipathic properties of lipids function in energy transducing particles such as chloroplasts, it does contribute significantly to fill the general void of knowledge in plant lipid metabolism. At the present time we have available considerable knowledge concerning lipid constituents in plant tissue but are largely ignorant concerning their metabolism.

In our studies with *Euglena* we have shown a pathway for de novo synthesis of long-chain fatty acids (18-20 carbonatoms) by a mechanism we consider to be a variant of the now classical malonyl-CoA pathway. The data suggest that the carbanions for condensation reactions are provided by the often suggested phosphoenolate of acetyl-CoA rather than malonyl-CoA. Bicarbonate, essential for the carboxylation of acetyl-CoA via the biotin containing acetyl-CoA carboxylase in the malonyl-CoA pathway

is not required in the Euglena system. Moreover, the Euglena system is not inhibited by avidin, the biotin-binding protein. From studies on stoichiometry, isotope dilution, and direct comparison of rates we have deduced the participation of the phosphoenolate of acetyl-CoA in this system.

Lipid Synthesis in Spinach Leaves⁽⁴⁾

The remarkable difference in distribution of GPG-and glycolipid to the chloroplast and N-containing phosphatides to other cellular particulates of greened leaves would suggest great differences in distribution of enzymatic activities associated with the synthesis of these two types of lipids. Furthermore, control mechanisms linked to chloroplast development probably are involved in the regulation of the synthesis of these "membrane" lipids, since a paucity of the glyco-and GPG-lipids exists in etiolated leaves.

Our preliminary study on lipid synthesis by particles from spinach leaves was confined to incorporation of C^{14} -1,3-glycerol-phosphate into lipid solubles. These studies covered the synthesis of phosphatidic acid, tri, di, and monoglyceride and probably GPG-lipid. Kinetics showed that phosphatidic acid was the precursor for all other lipids synthesized by a microsomal fraction. Although lipid synthesizing activity was observed in the chloroplast, its rate was only about 3 percent of the microsomal fraction. In contrast, the major activity for fatty acid synthesis in spinach leaves has been reported to be associated with chloroplasts. In terms of enzyme-chemistry these facts point out a great disparity in the cellular distribution of enzymes for lipid synthesis. This disparity, as well as the mechanism of galactolipid synthesis and the regulator controlling the chloroplast concentrated membrane lipids remain open to investigation.

Photoactivation of Photosynthetic Reactions in Dark Stored Bean Leaves

Results of Margulies and Jagendorf described a loss of photosystem II in bean leaves during dark, cold-storage and a subsequent photoreactivation of the O_2 evolving system. We have been attempting to gain some insight into the site of the lesion caused by this simple, mild treatment. Our investigations have been made both in vivo and in vitro in an attempt to more clearly delineate the problem. Since we found no quantitative differences in chlorophyll a or b, cytochromes, plastoquinone, total protein-nitrogen or total lipid content between normal and aged leaves, we looked for (possible) qualitative differences in the protein fraction. A qualitative difference may indeed exist since a protein fraction from normal active chloroplasts was found to restore O_2 evolving capacity. Although this observation has been made repeatedly, reproducibility left much to be desired and a great deal of effort will have to be made to define precisely conditions necessary for preparation of the protein.

No other in vitro treatment has been successful in restoring photosystem II. These treatments include poisoning the potential of the reactions with ferricyanide/ferrocyanide mixtures, additions of reductants, oxidants or sources of ATP. We reported previously (Quarterly Report) the restoration of ferricyanide reduction with α -tocopherol. This restoration was limited to ferricyanide reduction and not to O_2 evolution as judged by its lack of sensitivity to DCMU. In this connection, observations on the fluorescence induction in chloroplasts from aged leaves resemble closely the inhibition resulting from DCMU with normal chloroplasts. Thus the site of the lesion appears to be close to a primary event in photosystem II.

Mechanism of Action of Manganese in Photosystem II

Previous work by many has implicated manganese to be a unique component of photosystem II. Speculation of its role has been wide including the possibility that manganese is a part of a special chlorophyll.

Our work with uniformly labeled Mn^{54} Scenedesmus has given no evidence other than that manganese is bound to a protein of photosynthetically active particles. Under conditions of partial manganese deficiency during growth, 70-80% of the manganese supplied in the medium is concentrated and bound firmly to the protein of the particles. Studies to elucidate the binding groups of manganese on the protein are currently underway. This protein is difficult to bring into solution but progress has been made and purification is in the progress.

The restoration of deficient algae by manganese is light dependent and is not inhibited by uncoupling agents such as CCP. The requirement for light cannot be replaced by glucose.

During restoration (10 to 30 minutes) no increase in total protein nitrogen can be detected in contrast to normal cells. In this connection cycloheximide, a protein synthesis inhibitor, does not inhibit reactivation but does inhibit (90%) incorporation of C^{14} -phenylalanine into protein. These results suggest that within the cell the mangano-apo-protein is preformed. Requirement of light for the reactivation may indicate that a conformational change within the protein is necessary for the binding of manganese.

Photooxidation of Cytochrome c, Cytochrome f, and Plastocyanin⁽⁵⁻⁹⁾

The concept that photosynthesis is driven by two different photoreactions was supported by several earlier investigations at RIAS. Of the two photoreactions (systems I and II), system I is relatively easy to observe by itself and is more stable in cell free preparations. The initial photoact appears to be carried out by a special chlorophyll protein complex (P700), the absorption of a quantum resulting in an electron transfer from it to an unknown electron acceptor X (the primary reductant of photosystem I). The products of this initial charge separation are subsequently restored in dark reactions. P700⁺ (the primary photooxidant) regains an electron from associated electron carriers in the chloroplast matrix. Surprisingly, two such carriers seem to be operative, the first is cytochrome f, a heme component and the second is plastocyanin, a copper protein. Both carriers have a slightly lower normal potential than P700 and in both agents it is the metal which undergoes a valency change upon reduction or oxidation. The survival of these photooxidation reactions in detergent treated chloroplasts, and a more detailed study of the kinetics of these reactions have been reported in the quoted references.

Reducing Power of Photoact I^(10,11)

Our attempts to obtain information about the chemical nature of the primary photoreductant of photoact I (X) have so far been unsuccessful. As a speculation, we have proposed that this photoreductant (like the photooxidant) is a special chlorophyll molecule (so that the photoact would involve an electron transfer between two pigment molecules). This speculation is based on our attempts to obtain more information about the reducing strength of X⁻. In this work we used a number of viologen dyes of varying normal potentials and tried

to find at which potential substrate reduction no longer took place. It appeared that even the lowest viologen dye available to us ($E'_0 = 0.74$ volt) was photoreduced. Reductant X^- , therefore, must be located extremely low on the potential scale, lower than any presently known biological electron carrier.

Reducing Power Generated in Photosystem II⁽¹²⁾

Although the evidence is good that the photooxidant of system I P700 can be reduced by either cytochrome f or plastocyanin, it is as yet unclear which of these two components is involved under what conditions. Also, the question arises by which means these components are reduced in turn. One possibility we have considered, is that the two agents themselves are very close to the primary reductant(s) of photosystem II or even identical with it. More likely, however, the primary reductant of photoact II is a different agent with a somewhat lower redox potential. Speculations in the literature mention cytochrome b6 and plastoquinone, both with normal potentials around 0.0 volts. The availability of Scenedesmus mutant #8 isolated by Dr. N. Bishop provided a means to directly determine the reducing power of photosystem II. This mutant does not possess photoact I, and thus cannot produce low potential reducing power. However, it can reduce high potential substrates like ferricyanide (+ 0.43 volts) or benzoquinone, (+0.35 volts), reductions which are accompanied by the evolution of oxygen. Using cell free preparations of Scenedesmus mutant #8 and a number of different quinones with different normal potentials we determined the normal potential of the weak photoreductant of system II. The result was somewhat surprising: +0.18 volts, a value which at the moment cannot be correlated

with any known chloroplast intermediate. Also, if one assumes that this reductant in turn reduces cytochrome f , or plastocyanine (potential about + 0.37 volt) this electron transfer would barely suffice to allow the coupled generation of ATP from ADP. A different and possibly more incisive analysis of this problem has been recently undertaken. Rather than substrate reduction; fluorescence emission is now used to study the reduction properties of photoreductant II. After a number of experimental difficulties were overcome, we were able to confirm a normal potential of +0.18 volt. However, the situation proved to be more complex than we had expected, and further studies are indicated.

Fluorescence Studies^(13, 14)

During the last years, various aspects of the fluorescence emission of isolated chloroplasts and algae have been studied. One aspect, the time course of fluorescence upon a sudden onset of illumination has proven amenable to quantitative analysis. This analysis has revealed a number of quite interesting results. In essence, the fluorescence rise curve indicates the transformation of the primary reductant of photoact II into its reduced form, followed by the consecutive reduction of another (as yet unidentified) compound, present in equal concentration. The consecutive step occurs only at normal temperature but the primary conversion occurs even at the temperature of liquid nitrogen. It proved possible to "titrate" the number of primary acceptor molecules in the chloroplast, expressed in the number of chlorophyll molecules present. At the same time it was possible to determine the quantum yields of their reduction in photoact II and of their photooxidation by photoact I. If one assumes that in short wavelengths ($< 690 \text{ m}\mu$) the absorbed

light is equally distributed between the two photosystems, both primary processes proved to run with a quantum yield very close to unity. The number of primary reductants for system II proved of the order of one per 70 chlorophyll molecules. This implies that the "photosynthetic unit" (the number of chlorophyll molecules associated with a common reaction center) is much smaller in photosystem II than in photosystem I (one trapping center per about 500 chlorophylls). This "mismatch" between the two photosystems requires a peculiar arrangement of the intermediate electron carrier chain which connects photooxidant I with photoreductant II. This chain is by most people identified with the site of photophosphorylation. In addition to preliminary reports on these studies, three papers are in preparation which will give a detailed description.

Damaging Effects of Ultraviolet and Strong Visible Radiation⁽¹⁵⁾

If the light intensity is raised beyond the point where photosynthetic oxygen evolution is saturated (and in some plants even before this point) the photosynthetic apparatus is damaged. This results in a loss of quantum yield, and after prolonged exposure to complete destruction. To further understand this process and to localize its site of action, kinetic studies were made in the chloroplasts rather than whole algae. It proved that of the two photosynthetic systems photoreaction II is more easily destroyed. After photoinhibition, fluorescence emission is decreased and oxygen evolution stops. However, by adding ascorbate and indophenol to replace water as the ultimate electron donor, chloroplasts can still reduce TPN (system I operative). However, after prolonged excessive illumination, also photoreaction I is destroyed. In further analysis we compared the

activity of different wavelengths in this destruction. The interesting results showed that two processes were involved. The first one is largely a U.V. effect, the action spectrum shows a pronounced maximum of activity around 260 mμ and the activity drops very considerably in longer wavelengths. This U.V. process seems to be mainly responsible for the destruction of photoreaction II. A preliminary interpretation ascribes this destruction to the absorption of light by plastoquinone, this intermediate is often, but as yet inconclusively identified with the reductant of photosystem II. The second photoinhibition process, which occurs with a low quantum yield is sensitized by the same pigments (a.o. chlorophyll) which sensitize photosynthesis. Now both photoreaction I and II are destroyed, actually system I appears the most susceptible of the two. One difficulty which needs to be resolved, is that although the destruction by visible light is sensitized by the pigments of photosystem II, system I is the first to be destroyed. Forthcoming papers will describe the above in detail.

Mass Spectrometer Studies ⁽¹⁶⁻²⁴⁾

Our mass spectrometer technique which allows to measure directly the gases dissolved in a liquid by diffusion through a thin teflon membrane window has been used for two types of investigation in photosynthesis. The first one was an analysis of the rate of respiration in the light. By using O^{18} in the gas phase, oxygen taken up in respiration can be measured separately from O^{16} evolved from water in photosynthesis. Such measurements have been performed using a variety of conditions such as light intensity, and light color and with various algae. Most clearly in the blue-green algae, *Anacystis*, we found that in the light, respiratory oxygen uptake was suppressed to about

half the normal value. This suppression was most pronounced at long wavelengths which sensitized system I of photosynthesis. The most likely explanation is that in whole cells this system can provide cellular energy in the form of ATP. This can replace the ATP generated in darkness by the respiratory breakdown of storage materials. Light of shorter wavelengths, especially at higher intensities induces an abnormally high oxygen uptake. This process has not been explained satisfactorily. One possible speculation is that the extra uptake reflects an increased respiratory rate in strong light, which generates ATP, needed to assist the CO_2 reduction. This assumption rests on the idea that the ratio in which the photosynthetic light reactions generate reducing power and ATP is inadequate, more ATP being required than produced photochemically.

The second type of investigation with the mass spectrometer method concerned the exchange of oxygen by isolated chloroplasts. Whereas many substrates such as ferricyanide or TPN are reduced in the light with a concomitant evolution of oxygen, autooxidizable substrates like the viologen dyes can be reduced with a concomitant net oxygen uptake. This oxygen uptake results from the reoxidation of the reduced substrate, with the formation of hydrogen peroxide. The mass spectrometer differentiates between O^{16} evolved from water in photosynthesis and O^{18} consumed from the air in the recombustion. This exchange reaction, analyzed first by Mehler, occurs in chloroplasts even in the absence of external substrate. In some chloroplast preparations the rate can amount to one third the rate observed in the presence of a mediator. One of the problems we are pursuing at present, is which type of natural component in the chloroplasts is responsible for this exchange reaction. The first question to be asked is whether both photoreactions

are involved in this exchange or only photosystem II. Finally, the mass spectrometer has been used in conjunction with other methods (notably the measurement of TPN reduction) for a further analysis of action spectra and enhancement effects.

Identification of the Sharp Light Induced Electron Spin Resonance Signal with Photo Converter P700^(25,26)

In collaboration with Dr. H. Beinert (Univ. of Wisconsin) we have tried to measure the number of spins involved in the typical narrow light induced signal and to correlate this with the number of P700 molecules. Both quantitative determinations are beset with experimental difficulties. The data showed excellent qualitative correlations between the two phenomena. However, the numbers obtained in quantitative determinations indicated a ratio of 2 spins/P700 instead of one, as we had expected. As yet, it remains an open question whether experimental uncertainties are responsible for this discrepancy or an as yet understood mechanism. An EPR instrument recently became available at RIAS and we will attempt to improve its sensitivity to a sufficient degree to allow a renewed attack on the problem.

Miscellaneous

A number of other projects have been going on in our photosynthesis group, some of which have been discussed in our earlier quarterly reports. Studies of photosynthetic electron transport by means of fast and sensitive difference spectroscopy and development of appropriate techniques constituted a major fraction of our effort^{27,28}. Studies concerning the process of photophosphorylation and of the light potentiated ATP hydrolysis²⁹ were not further pursued after the departure of Dr. Hoch. A renewed investigation of

the effects of wavelength upon photophosphorylation and various modes of electron transport by which it is mediated, is presently being pursued by Dr. Schwartz. The peculiar alga *Ochromonas* and cell-free preparations of it have been given considerable attention but as yet the observations are difficult to interpret. Finally, we should mention a conference on photosynthesis sponsored by the National Academy of Science which was chaired by one of us, as well as a few general review articles written by members of the photosynthesis group³⁰⁻³³.

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